MICROMACHINED STIMULATING MICROELECTRODE ARRAYS

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MICROMACHINED STIMULATING MICROELECTRODE ARRAYS

Summary

This contract seeks to develop a family of thin-film stimulating arrays for use in neural prostheses. STIM-2B/-3B are two- and three-dimensional arrays of stimulating sites on 400µm centers. The probes have four channels and 64-sites. Any selected site can be used for either recording or stimulation. Current generation is off-chip. The high-end probes STIM-2/-3 are similar except they use on-chip current generation via 8-bit digital to analog converters.

During the past term, we have continued a look at TiN as a possible stimulating electrode site. While for short pulses, it has an impedance comparable, and even slightly lower, than IrO sites of comparable size, it does not appear to provide superior performance overall. Some studies of both TiN and polypyrrole will continue in the recording area, however. Histology has been performed on probes implanted in guinea pig for three weeks. Immunocytochemistry with antibodies to cell specific proteins were utilized to improve our characterization. Glial Fibrillary Acidic Protein, a marker of glial cells, showed immunostaining around the probe tracts, confirming earlier observations that glial cells comprise an element of the tissue envelope. Vimentin immunoreactive labeling around probe tracks was also observed in many animals, indicating that epithelial cells can also be a component of the tissue envelope, likely due to cells migrating down the probe track from pia and/or pial cells pushed down during the probe insertion. Fibronectin (an epithelial matrix protein) immunolabeling was asymmetric on only one side of the probe and is being explored further.

With working versions of STIM-2B/3B now in hand, work is concentrating on the external interface required to operate them in detailed and automated mapping studies between the dorsal cochlear nucleus and the inferior colliculus in guinea pig. The goal is to be able to acoustically stimulate cells in DCN and record from the cells they drive in IC, then repeating this using electrical stimulation of the same cells in DCN. We have improved the graphical user interface (GUI) for the probes so that we can produce the multi-channel signals necessary to these mapping studies along with the required software. We have also developed the cabling and external drive circuitry to enable the experiments to proceed. We hope to carry out the first such experiments during the coming term. Circuit iteration on STIM-2 and STIM-3 is also now underway.

MICROMACHINED STIMULATING MICROELECTRODE ARRAYS

1. Introduction

The goal of this contract is the development of active multi-channel arrays of stimulating electrodes suitable for studies of neural information processing at the cellular level and for a variety of closed-loop neural prostheses. The probes should be able to enter neural tissue with minimal disturbance to the neural networks there and deliver highly-controlled (spatially and temporally) charge waveforms to the tissue on a chronic basis. The probes consist of several thin-film conductors supported on a micromachined silicon substrate and insulated from it and from the surrounding electrolyte by silicon dioxide and silicon nitride dielectric films. The stimulating sites are activated iridium, defined photolithographically using a lift-off process. Passive probes having a variety of site sizes and shank configurations have been fabricated successfully in past contracts and have been distributed to a number of research organizations nationally for evaluation in many different research preparations. For chronic use, the biggest problem associated with these passive stimulating probes concerns their leads, which must interface the probe to the outside world. Even using silicon-substrate ribbon cables, the number of allowable interconnects is necessarily limited, and yet a great many stimulating sites are ultimately desirable in order to achieve high spatial localization of the stimulus currents.

The integration of signal processing electronics on the rear of the probe substrate (creating an "active" probe) allows the use of serial digital input data which can be de-multiplexed on the probe to provide access to a large number of stimulating sites from a very few leads. Our goal in this area is to develop a family of active probes capable of chronic implantation in tissue. For such probes, the digital input data must be translated on the probe into per-channel current amplitudes that are then applied to the tissue through the sites. Such probes generally require five external leads, virtually independent of the number of sites used. As discussed in previous reports, we have designed a series of active probes containing CMOS signal processing electronics. Two of these probes have been completed and are designated as STIM-1A and STIM-1B. A third probe, STIM-2, is now ready for a final iteration and is a second-generation version of our original high-end first-generation design, STIM-1. All three probes provide 8-bit resolution in digitally setting the per-channel current amplitudes. STIM-1A and -1B offer a biphasic range using ±5V supplies from 0µA to ±254µA with a resolution of 2µA, while STIM-2 has a range from 0 to ±127µA with a resolution of 1µA. STIM-2 offers the ability to select 8 of 64 electrode sites and to drive these sites independently and in parallel, while STIM-1A allows only 2 of 16 sites to be active at a time (bipolar operation). STIM-IB is a monopolar probe, which allows the user to guide an externally-provided current to any one of 16 sites as selected by the digital input address. The high-end STIM-2 contains provisions for numerous safety checks and for features such as remote impedance testing in addition to its normal operating modes. It also offers the option of being able to record from any one of the selected sites in addition to stimulation. It will be the backbone of a multi-probe three-dimensional (3D) 1024-site array (STIM-3) now in development. A new probe, STIM-2B, has recently been added to this set. It offers 64-site capability with off-chip generation of the stimulus currents for four separate channels. These channels are organized in four groups so that each current can be directed to any of the 16 sites in its group. Each selected channel can be programmed for either stimulation or recording. On-chip recording amplifiers offer a gain of 50; alternatively, the neural activity can be recorded using off-chip amplifiers

interfaced through the normal stimulating channels. This probe is available in both 2D and 3D versions (as STIM-2B/3B) and is now being used in-vivo.

During the past quarter, we have continued to fabricate passive probe structures for internal and external users and have also performed histology on some additional stimulating probes. With fully functional versions of both STIM-2B and 3B now available, we are also working on the iteration of our external probe interface to support in-vivo mapping studies that will demonstrate the utility of this active probe family. This work involves both hardware and software additions to the probe interface. The results in each of these areas are described more fully in the sections below. In addition, it should be noted that during the past six months we have had considerable turnover in the student personnel active in this project and the companion Thin-Film Intracortical Recording Microelectrode project. Tracy Bell, who explored the development of alternative micromachining processes for probe fabrication and developed a prototype cochlear stimulating probe to validate the process, graduated and is now employed at Hewlett-Packard Corporation. Ying Yao has now joined the Stimulation project to complete the development of STIM-2/3.

2. Passive Probe Developments

During the past quarter, four additional wafers were etched out from the STANDARDS mask set. This mask set includes a variety of single- and multi-shank stimulating and recording probes with and without integrated cables that comprise the CNCT Passive Probe Catalog. Two wafers have standard iridium sites to supply users of the CNCT distribution service, one has gold sites, and the final has TiN sites.

The wafer with gold sites was fabricated for several collaborators including Ross Meyer and Stuart Cogan of EIC Labs, and Tracy Cui and David Martin of the University of Michigan. As described on a poster at the recent NIH Neural Prosthesis Workshop,[1] EIC is performing studies on electro-deposited iridium oxide as a possible electrode site interface. This type of film may have several advantages over our current iridium sites in that the post-processing may be simpler to carry out than actual activation, and gold deposition is more foundry-compatible than iridium deposition, even though neither is very commonly used in the integrated circuit industry. Also, as described in an NIH Neural Prosthesis Workshop poster,[2] work is being done at the University of Michigan to characterize polypyrrole as a potential site interface for chronic electrodes. Polypyrrole not only provides a more convex surface, which may encourage mechanical cleaning of the site during small natural movements of the brain, it can also be instrumented with bioactive molecules to improve the site/cell interface. Gold sites may provide a better surface than iridium on which to deposit the polypyrrole, at least for preliminary experiments, since gold does not have its own redox reaction and therefore will not complicate the electrochemical measurements of polypyrrole.

This second run of TiN probes was completed so that additional *in vitro* and *in vivo* characterization and verification of the site properties could be performed. As described in previous reports, TiN is being studied for its potential use as a stimulation and/or recording site[3]. In addition to having properties that may make it a useful material for high frequency

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[1] R.D. Meyer, T.H. Nguyen, R. Rauh and S.F. Cogan, " Electrodeposition of Iridium Oxide Charge Injection Electrodes"

[2] X. Cui and D.C. Martin, "Surface Modification of Neural Prosthetic Devices by Conducting Polymer Polypyrrole"

[3] F. Yuan, J. Wiler, K. Wise and D. Anderson, "Micromachined multi-channel Microelectrodes with Titanium Nitride Sites" *Abstracts, Joint meeting of the BMES and EMBS*, Atlanta, Oct. 1999.

stimulation, TiN has a lower impedance at mid-range frequencies than pure iridium which may make it useful for at least some types of recording studies. Unlike sputtered pure iridium, TiN does not require post-processing to lower its impedance (iridium must be electrochemically activated). A typical 175μm^2 unactivated iridium recording site has a 1kHz impedance of 2-4MΩ. A comparable TiN site is at least an order of magnitude lower in impedance (~300kΩ) and is similar to activated iridium.

As a stimulating electrode material, neither TiN nor polypyrrole may be preferable to IrO; however, for recording both have advantages and are being pursued as described in the companion report on Thin-Film Intracortical Recording Microelectrodes.

3. Histological Studies of Implanted Probes

We have recently gathered new and interesting data about the envelope of tissue reaction surrounding neuroprobes following three weeks of placement in the guinea pig cortex. Immunocytochemistry with antibodies to cell specific proteins were utilized to improve our characterization. Glial Fibrillary Acidic Protein, a marker of glial cells, showed immunostaining around probe tracts (Fig. 1), confirming our earlier observations that glial cells comprise an element of the tissue envelope. More surprisingly, vimentin immunoreactive labeling around probe tracks (Fig. 2) was also observed in many animals. Vimentin is an intermediate filament protein specific to epithelial cells of mesenchymal origin. Thus, epithelial cells can also be a component of the tissue envelope. Their source could either be from pierced blood vessels or the pierced meninges. When endothelium specific antibodies were applied, no labeling around tracts was observed, suggesting the epithelial cells were not from blood vessels and therefore most likely to be cells migrating down the probe tract from pia and/or pial cells pushed down during the probe insertion. Another interesting observation in several animals was Fibronectin (an epithelial matrix protein) immunolabeling (Fig. 3). This was asymmetric, only one side of the probe and could be related to movement of the probe in the brain.

4. Active Stimulating Probe Development

The active stimulating probe development has progressed into the long-term testing stage of the STIM-2B probes. We are preparing to use the STIM-3B probe arrays in some simple mapping experiments. The user interface system software has been upgraded and some versions of the cable terminator/cable connector have been fabricated.

STIM-2B

STIM-2B is a second-generation version of our simplest active stimulating probe, STIM-1B. STIM-2B has extended the single-channel 16-site STIM-1B probe to four independent externally-routed current-stimulus channels with 64 sites on 16 shanks. Each channel is routed through a 1-of-16 selector. The design has been successfully fabricated in our standard bulk-micro-machined CMOS process. The functionality of the digital portion of the circuitry has been verified through testing of the different modes of the probe: POR (activation mode) site selection (stimulation mode), amplifier-on, etc. The amplifier has been demonstrated to work as well in-vitro, but when tested in-vivo, operation has been problematic due to large DC drifts of the iridium site-tissue interface potential. This is being addressed through the development of appropriate devices for input dc stabilization.

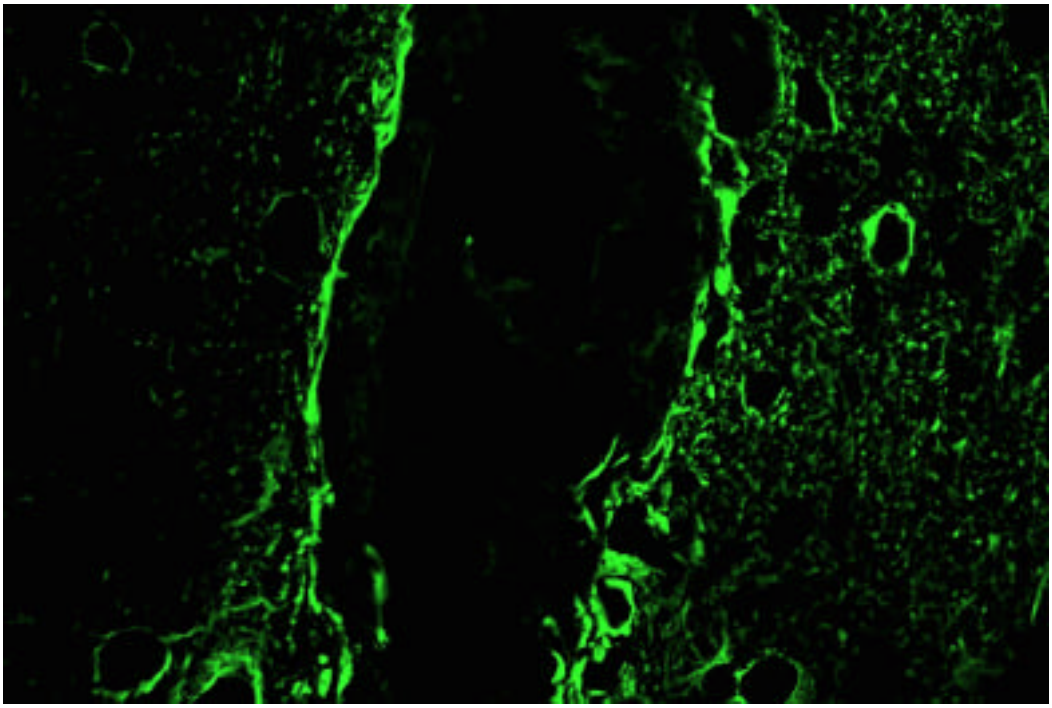


Fig 1: GFAP marker around an electrode track.

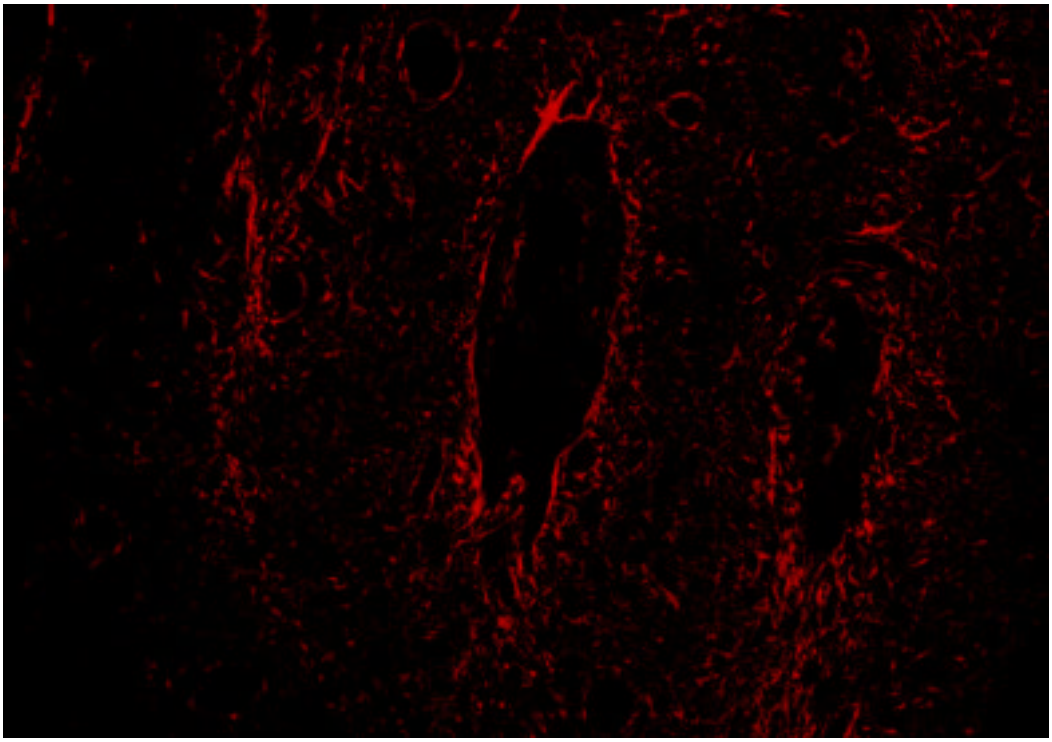


Fig. 2: Marker for Vimentin, an intermediate filament protein specific to epithelial cells of mesenchymal origin.

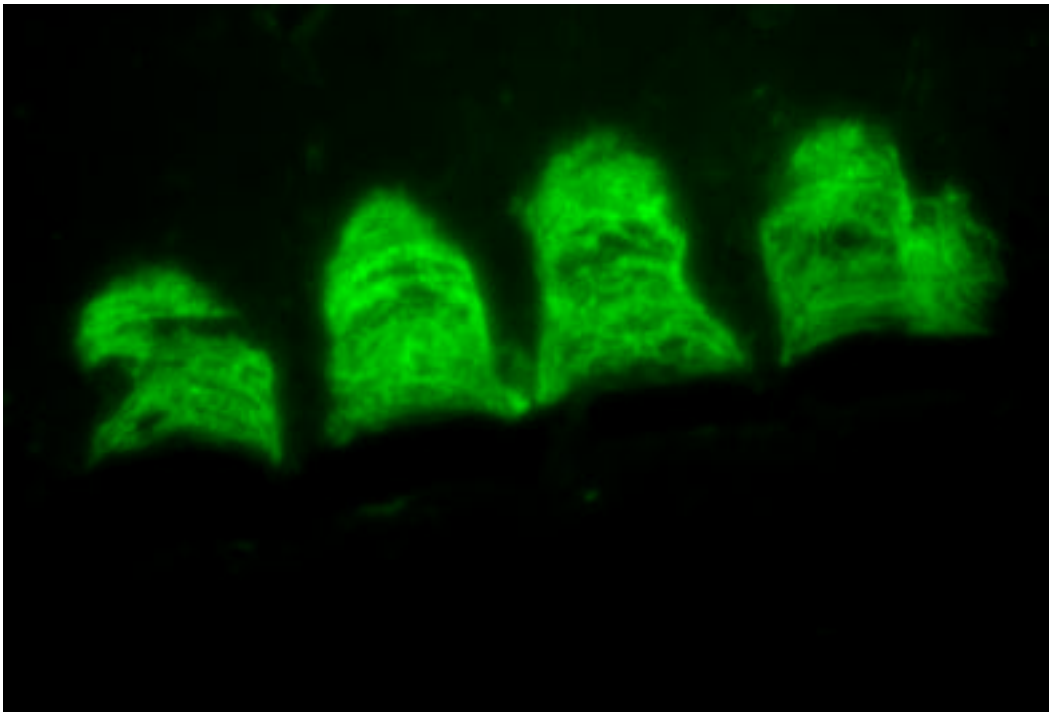


Fig. 3: Marker for Fibronectin (an epithelial matrix protein) shows an asymmetric distribution around 4 probe shanks.

The capabilities of the STIM-2B probe have been demonstrated to be an important tool for performing some interesting and important experiments by allowing stimulation access to a relatively large area of neural tissue without mechanical repositioning of the probe. This capability is realized by utilizing a 20b shift register to load four 4b site addresses which are decoded by a 1-of-16 nand-type decoder to connect the desired site to an analog input/output pad through a large CMOS passgate transistor thereby allowing the 'steering' of externally generated currents to the addressed site. A recording function was included on this probe and is addressed by a fifth bit included with the 4b site address. This fifth bit selects between stimulation mode and recording mode by selecting either a direct path to the I/O pad from the site or a path through an amplifier for recording from the same site. Each I/O channel has its own dedicated amplifier so that the functionality of all of the channels is essentially independent of each other except for the up-front data input circuitry.

We have been using the STIM-2B probes in acute experiments for both stimulation and recording. Because of the problem we have had with the operation of the on-chip amplifiers *in-vivo*, we have only been able to perform successful recording with the probes in the direct-access or stimulation mode. In spite of this sub-optimal performance, we have been able to very successfully record single-unit activity, both spontaneous and acoustically driven. The two capabilities of stimulation and recording combined on one substrate are what we feel make this probe such a valuable tool in neuroscience research and as the basic building block for future neuroprostheses. Some examples of recorded responses are shown below in Figures 4-6. Figure 4 is a typical recording obtained through an unbuffered stimulation channel of a STIM-2B probe while recording in IC with acoustic stimulation provided by a 100mSec noise burst. Figure 5 is the post stimulus time histogram (PSTH) of spike counts at the same site. The two PSTH's of Figure 6 are of

interest because they were recorded simultaneously from two sites on adjacent shanks separated by 400µm. The responses are not being recorded from the same cell because of the large site separation (this was verified from the raw signals), but the responses are very similar in shape with the one response simply being delayed relative to the other. From these PSTH's one can see that temporal processing can be relatively easily investigated with the STIM-2B probe. We have also attempted to perform simultaneous electrical stimulation and recording using two STIM-2B probes, one stimulating in the DCN and the other recording in the IC, but the logistics of using two active probes simultaneously has been challenging and success has thus far been limited. We expect this milestone to be accomplished in the near future as we become more proficient in the setup, use, and synchronization of two separate probe systems.

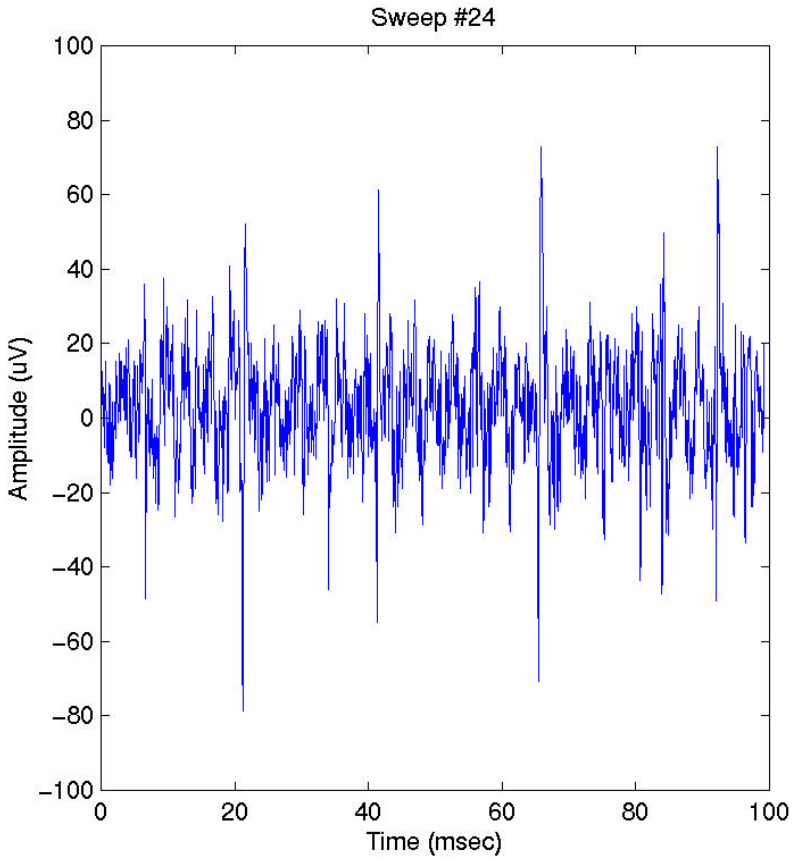


Fig. 4: Accoustically driven neural signal recorded through a STIM-2B probe in the IC.

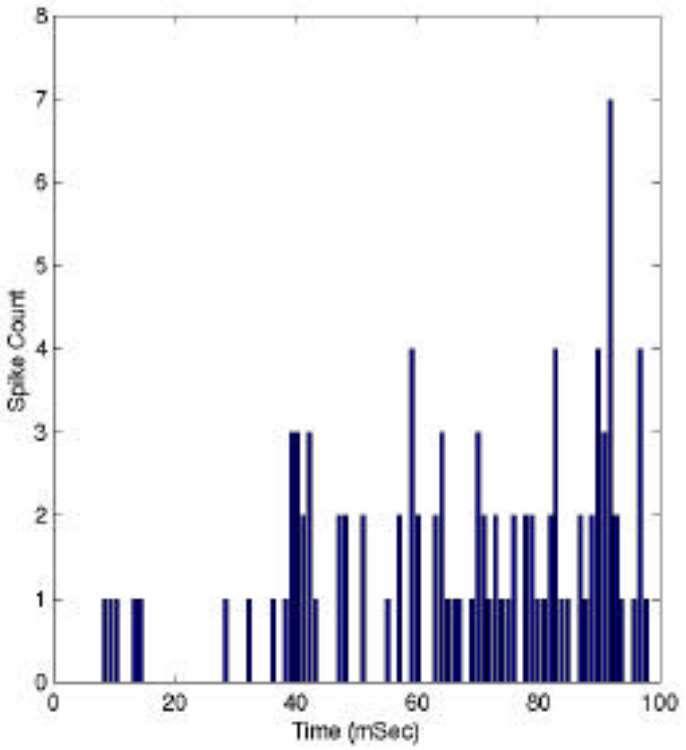
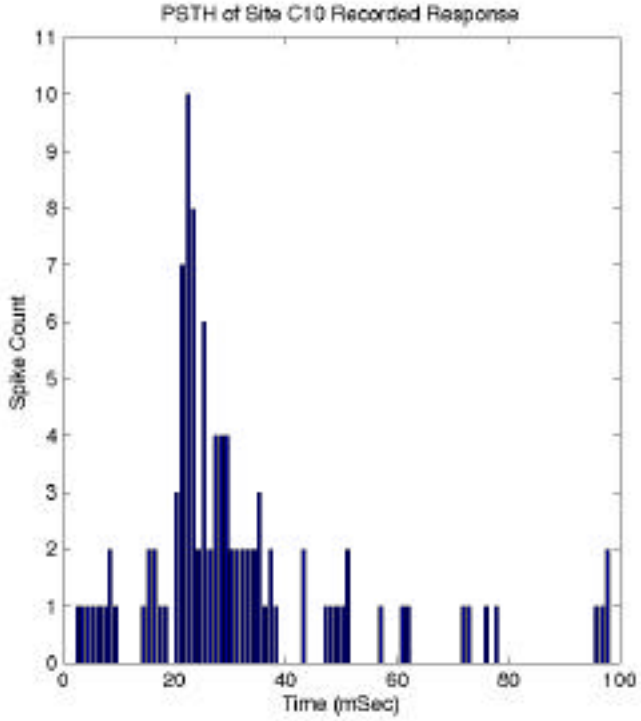
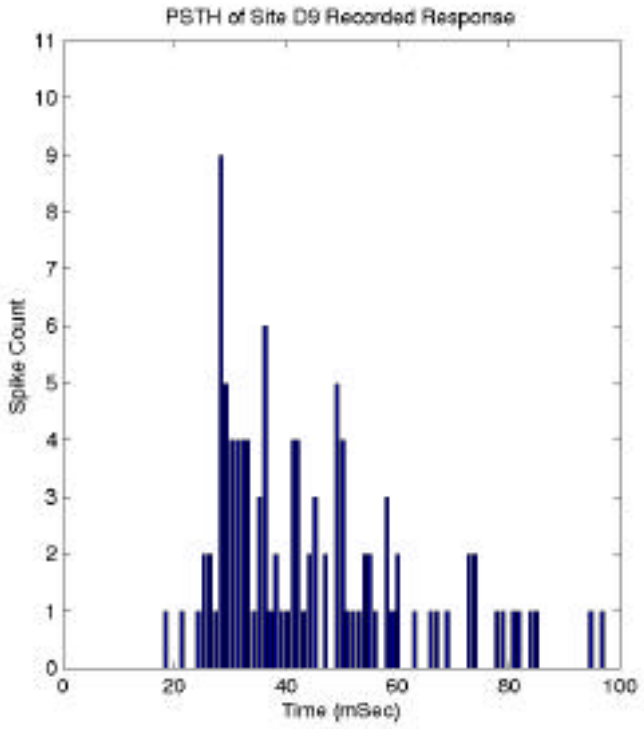


Fig. 5: PST histogram of recorded neural spike count in IC during acoustic stimulation.



a)



b)

Fig. 6: Acoustically-driven responses recorded in IC where the site in a) is on one shank and the site in b) is 400µm away on the adjacent shank.

The current work with the STIM-2B probes is additionally moving into long-term *in-vitro* testing. Several versions of the cable terminator/probe connector, as discussed in depth in the previous report, are completed (including recording-only or stimulation-only versions) and we are using them to start long-term pulse testing to see that the encapsulation layers, the iridium sites, and the circuitry all perform as expected in a saline environment over extended periods of time. In order to observe any changes in the iridium sites, we observe the sites which will be pulsed by taking surface profile scans with a Zygo interferometer, optical and SEM photographs, and CV measurements before starting pulse testing and at intervals during the testing. We also monitor the probe power supply lines during testing in order to observe any current leaks that might occur whether through the dielectric encapsulation layers or through the epoxy used to encapsulate the bonding wires.

We are doing some comprehensive comparisons of the site activation techniques that we have tested on a small scale up to this point by looking at parallel activation of all sites versus serial activation of individual sites. This comparison is being performed by activation of sites in parallel and then observing the individual CV characteristics of the sites and see how much variation there is across the entire array of sites. This experiment does require new probes for each test, so more probes are being etched out to increase the sample size for this experiment (though they can and will be used for future *in-vivo* experiments after measurements have been made). We have used parallel activation with good success in a limited number of cases as reported previously, but it would be better to have a larger number of samples to confirm our results. The CV plots that we have previously reported were somewhat noisy due to the rats-nest of wires required to operate a probe within the CV measuring system. By consolidating the user interface system to a

relatively small printed circuit board and making improvements in lead shielding, the new external user interface system has proven to significantly reduce the noise coupled into the measurements, as can be see below. The CV plot shown in Figure 7 is data measured while using the wire-wrapped prototype of the user interface system. The CV plot shown in Figure 8 is data measured while activating the iridium sites of a STIM-2B probe in preparation for a recent experiment.

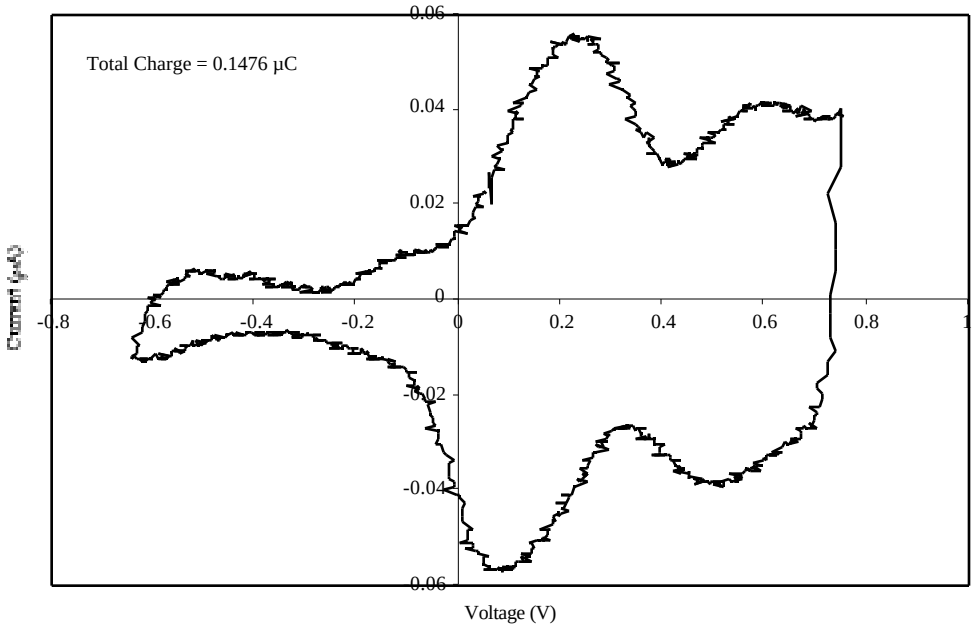


Fig. 7: An example of noisy CV curve previously measured while using the prototype version of the external interface system to operate the probe.

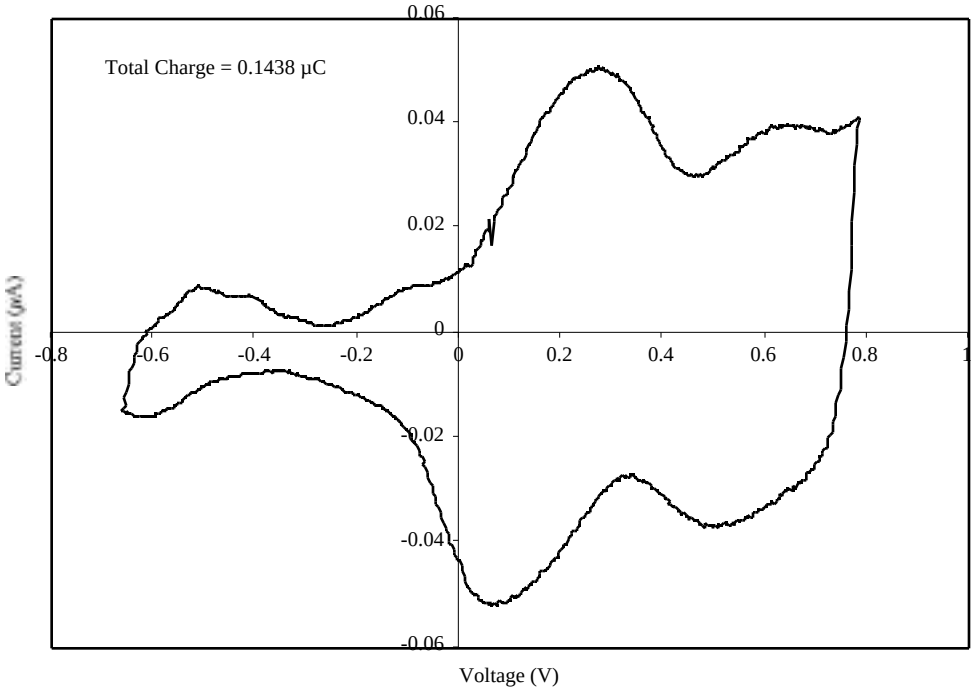


Fig. 8: A typical CV curve measured while operating the STIM-2B probe with the new PCB version of the external interface system with well-shielded cables.

We are also looking at the effect of repeated use of the same probe for multiple experiments and the effect that this has on the CV characteristics of the sites. In preparation for previous *in-vivo* experiments, we have noticed some changes in the CV characteristics in used probes and we would like to know the cause. Though this is not known for sure yet, it may be simply due improper cleaning after each and may indicate a more rigorous cleaning procedure is required after each use.

STIM-3B

The logical extension of STIM-2B is to make it into a three-dimensional (3D) array, which is exactly what we did to realize STIM-3B. STIM-3B is set up in a platform configuration with an integrated ribbon cable for connection to a percutaneous plug to allow use as a device in chronic experiments. The differences are some structural modifications to allow interconnection to a 3D-platform assembly and a few more minor circuit blocks to facilitate the addressing of multiple probes in a 3D array.

The functionality of the STIM-3B probe is expected to provide an important tool for performing some very important and interesting experiments by allowing the acute and chronic stimulation access to a relatively large volume of neural tissue without mechanically repositioning the probe. As in the STIM-2B design, the STIM-3B probe is a four-channel, 16-shank, 64-site probe which routes four externally generated stimulus signals to 1-of-16 sites per channel. This capability is realized by utilizing a 20b shift register to load four 4b site addresses which are decoded by a 1-of-16 nand-type decoder to connect the desired site to an analog input/output pad through a large CMOS passgate transistor thereby allowing the steering of externally generated stimulus currents to the addressed site. A simple recording function has been included and is addressed by a fifth bit included with the 4b site address. This fifth bit selects between stimulation mode and recording mode by selecting either a direct path to the I/O line from the site or a path through an amplifier for recording from the same site. Each I/O channel has its own dedicated amplifier so that the functionality of all of the channels is fairly independent of each other except for the up-front data input circuitry.

In order to allow addressing of multiple probes in a 3D array, STIM-3B has an extra 4b serial input shift-register which, when the bits are set, connects the corresponding I/O channel of the probe onto a common I/O bus on the platform. All of the extra registers of the probes in a STIM-3B array are connected in series via platform leads to form an extended or virtual register. The virtual register enables all of the probes of a 3D array to be addressed with only two address lines, a channel enable address line and a site address line. This architecture does have a limitation in that it does not allow independent use of the same site address on two separate probes in the array. The slightly reduced flexibility was considered a reasonable sacrifice to achieve a reduction in circuit complexity and more importantly lead count.

Rather than waste area on the active CMOS probe mask set, the STIM-3B 3D structural pieces were designed on a completely separate mask set which only required six masks and was essentially the same fabrication process as is used for passive probes. The only difference being that there are no site or pad masks, instead, there is a single beam/pad electroplating mask. The electroplating mask is used as the final step to form the beam lead interconnects and pads on the platforms after the field etch has been done. The conductor mask was designed with the polarity reversed from what it would normally be for a passive probe so that it could be used in a lift-off process for metal conductors instead of

polysilicon. The first platforms were fabricated still using polysilicon as the interconnect (patterned by image reversal), but we anticipate using lower resistance metal interconnect layers on future platforms.

We have several different platform designs that were fabricated: a 16-probe platform and three 4-probe platforms. The 4-probe platforms include: a version that has all probes facing the same direction, a version that has all probes facing towards the centerline and a version that has all the probes facing the same direction but with the ribbon cable exiting from the side of the platform instead of the end as on the others. The platforms all have integrated microfuses to allow the number of probes included on the platform to be varied. If a fuse is not 'blown', the data will propagate to the next probe slot without needing to be routed through the probe that would have been in the current slot.

We are currently preparing to use these probes first in acute experiments, but then hopefully in chronic experiments. The main difficulty in using these probes is the sheer size of the arrays relative to the model animal that we typically use, the guinea pig. The guinea pig brain is almost not large enough to accommodate such a large structure. We are currently looking at ways of successfully performing chronic implants of these 3D arrays. Initially, only the four probe arrays will be used because the 16-probe array would be so large as to be basically unusable in the guinea pig. There are still some issues in realizing the STIM-3B probe arrays in large quantities. The bonding of the beam leads is a task that we are still working on improving because of the difficulty in making the bond without damaging the platform or probes. We will also need to complete more wafers of active probes because of the number of probes required to populate numerous arrays.

5. External Stimulating Interface System

We have designed and constructed an interface system for active stimulating probes, as described and shown in previous reports. The printed circuit board realization of our hardware design has been fabricated and tested. This version of the system operates much faster and supports a probe bit rate at up to 9.5Mbits per second and down to a division by 256. The system is operated remotely via a serial cable link to a personal computer. Graphical software has been developed for easy use of the system and a command-line interpreter is included in a separate window, which allows for fine-grain control over the remote system. Whereas the graphical component of the interface is intended as an easy-to-use method for performing common functions, the command-line interpreter can be used to effect any other function not supported by the graphical interface.

We have upgraded the graphical user interface (GUI) and digital signal processor (DSP) operating system software to allow the user to independently control (via the GUI) the four current channels of STIM-2B and have the user interface system handle the commands correctly and efficiently. The user now has the capability to independently change the amplitude and duration of each phase of a biphasic pulse on each of the four channels. A pulse delay can be specified so that the pulse on any channel can be independently delayed relative to the other channels. A delay can also be specified between phases of the biphasic pulse. At this point of development the repetition rates of all channels must be the same, but we are also looking into making this parameter independent as well if such a mode is deemed useful. The GUI window is shown below in Figure 9. Figure 10 demonstrates the output of the system monitored on an oscilloscope when running with the shown parameters. Note that the observed output is simply the voltage signal that is used to drive the voltage-to-current converter. Comparison of the expected waveforms in Figure 9 with those actually realized in Figure 10 reveals that the interface

system is working as expected. The waveforms of the oscilloscope trace are not the same relative widths as the GUI displays because the GUI displayed waveforms are automatically scaled to fill at least 1/3 of the display window in order to make it easier to see the waveform shape. We anticipate continuing to upgrade the functions of the GUI as we find things that we feel would be useful as a result of our using this system for experiments. This aspect of development is very important in that it is difficult to foresee which features are useful without trying to actually make use of the system under typical experimental conditions.

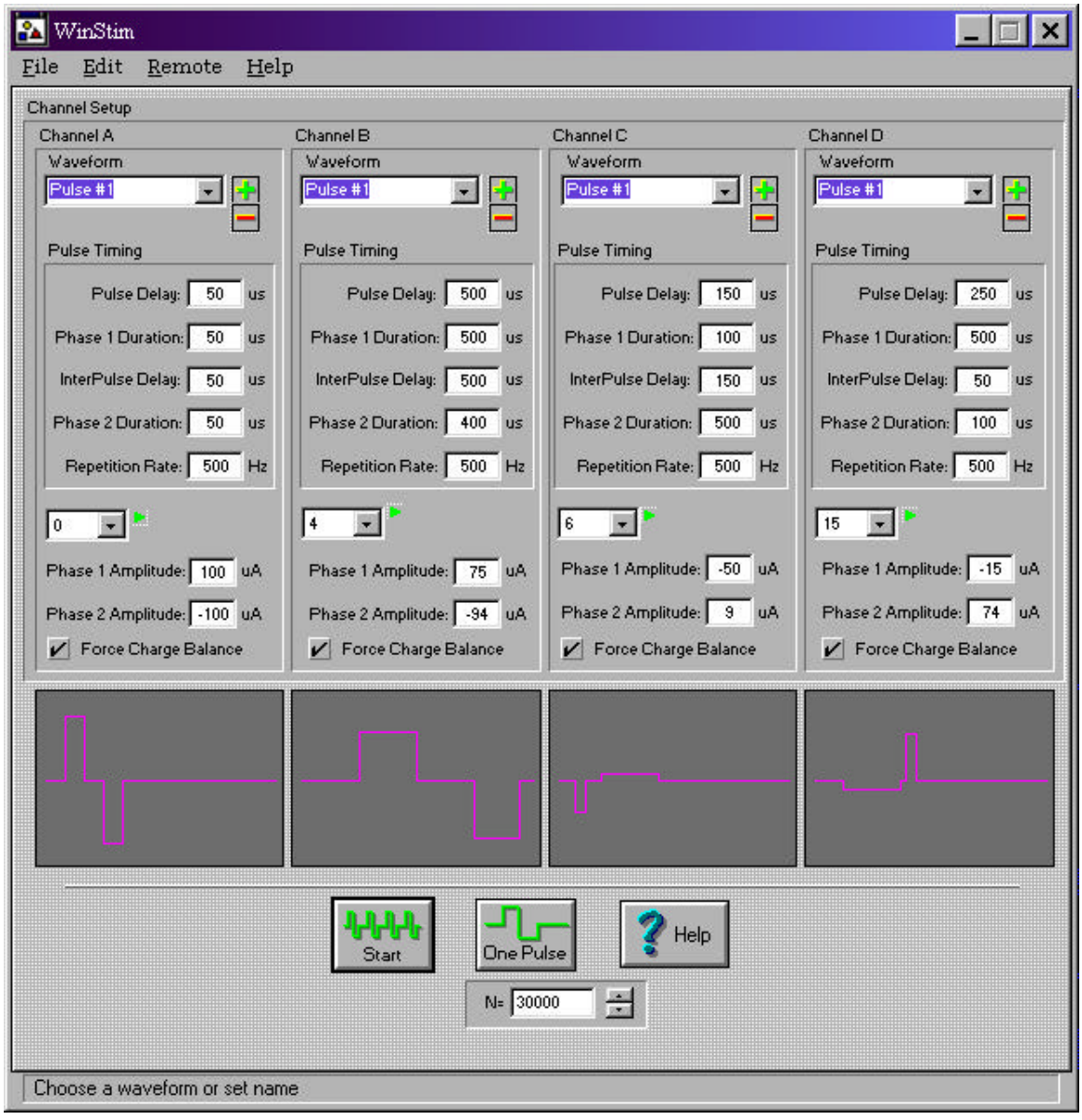


Fig. 9: The GUI window of the user interface window. Note that the time-scales of the waveform displays are not necessarily the same because each is automatically scaled so that the waveform is not less than 1/3 of the viewable window.

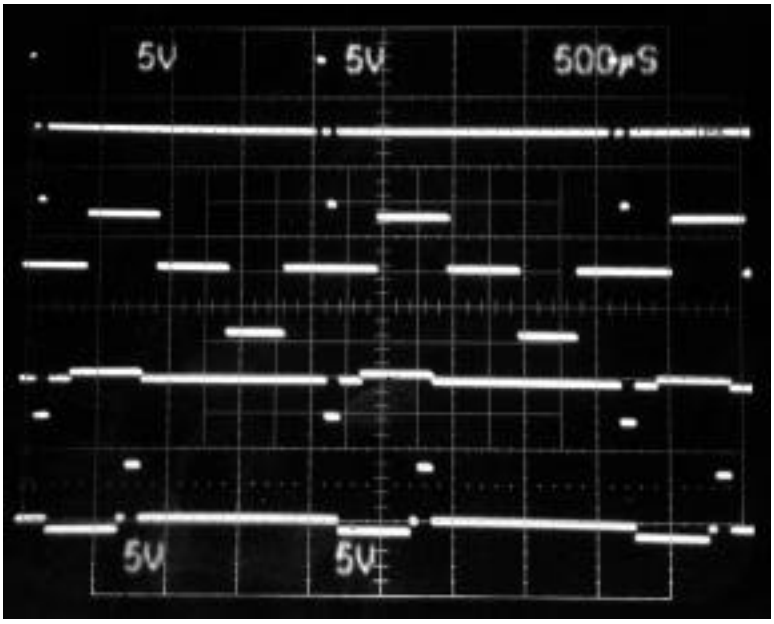


Fig. 10: An oscilloscope trace of the four channels of voltage output of the system (channels A-B, top to bottom) which drive the voltage-to-current converters. Note, these traces were generated with the setup of Fig. 9.

In testing the prototype board, it was found that the a standard serial cable used to connect the output of the printed circuit board to the input of the probe did not provide as much cross-talk suppression as we needed since the digital and analog lines all run through the same cable. A solution to the problem was to utilize a Sun Video Cable as the cable connecting to the location of the probe. The Sun Video Cable has three shielded mini-coax lines, see the Video Cable connector in Figure 11, which are used for the high speed data and clock lines and the remainder of the signals are carried over shielded twisted-pair lines. As discussed in the previous quarterly progress report, the use of the Sun Video Cable has solved several problems, but we have also found two more problems when used with the STIM-2B/3B probe design, and we are in the process of correcting them. First, we needed to design and fabricate a cable termination/probe connector board, though some version of this would have likely been necessary no matter what cable were used. Secondly, a problem arose from the need to externally drive current stimulus pulses. The parasitic shunt capacitance of the shielded cable diminishes the capability to deliver short, precisely controlled current pulses to the probe site.

The custom interface board includes a D/A converter with a voltage output. The board was designed with the capability to plug in a voltage-to-current converter daughter card. The reason for this was because of the difficulty experienced in designing a circuit with current monitoring feedback that will perform with the response times that are desired. The plug-in daughter board design allows the voltage-to-current converter to be easily upgraded as the design is improved. Thus, the current configuration makes it relatively easy to solve the problem of the parasitic shunt capacitance. Since a cable termination/probe connector is needed anyway, the voltage-to-current converter is being pushed out onto the connector where it is much closer to the probe and the cable shunt capacitance can be quickly charged with a high-current voltage driver.

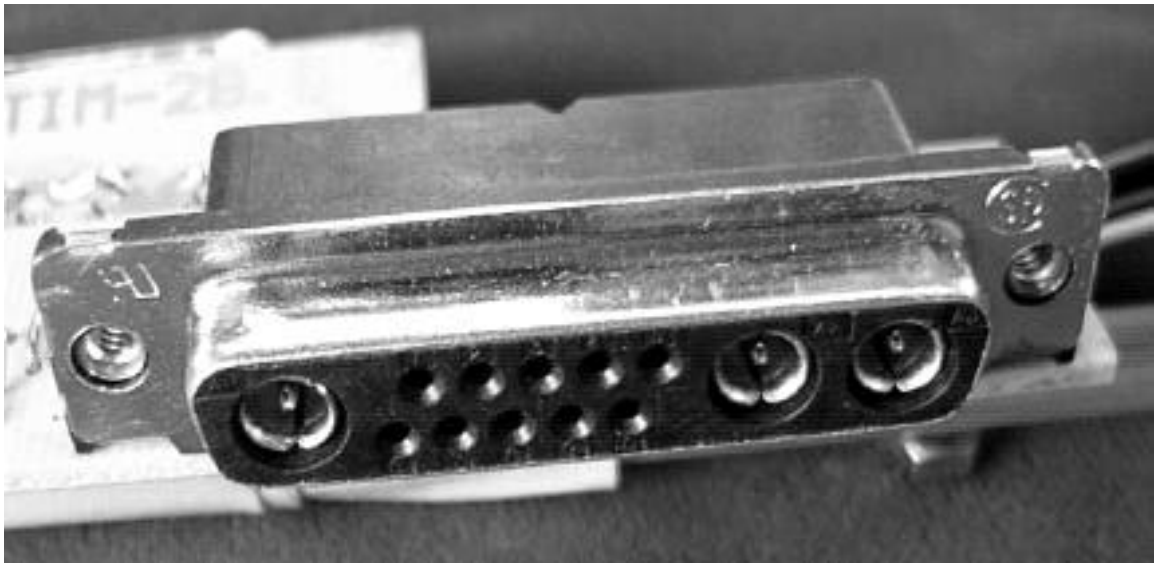


Fig. 11: A close-up of a Sun Video Cable connector mounted on the PCB cable terminator/probe connector. The 3 mini-coax are used to route the high-speed digital signals (Clock, Y-Data and X-Data) to the probe.

The circuit on the connector has evolved with the addition of several other blocks being added for various functions. An amplifier for monitoring the power supply current is being included. Circuitry giving the capability to record from certain stimulating probe designs is also being included on the connector. The block diagram for the entire connector circuitry is shown in Figure 12. The entire system has not yet been built on a single PCB. We have fabricated several PCB's in-house with portions of the circuitry included as shown in Figure 13 so that these can be used to perform the various tasks needed for experiments. As can be seen in Figure 13, a completed PCB is bolted to a dowel, which is then mounted onto a manipulator. The center board has a four-channel unity-gain buffer amplifier that was used for the recordings that were done with STIM-2B. The lowest PCB is shown such that the Sun Video cable connection and the dip socket for probe mounting can be seen. The dip socket has proved to be somewhat problematic in that the mechanical force required to insert and remove the probe stalk was high enough to cause some difficulty during experiments. Methods of alleviating this problem are being evaluated. All of the circuit components of the unity-gain buffer amplifiers were surface mount packages so that the PCB can be made as small as possible, see Figure 14. We plan to do the same for the complete connector PCB and it will additionally be enclosed in a metal case to provide environmental and electrical shielding. A PCB connector with a probe stalk (and probe) attached is shown in Figure 15.

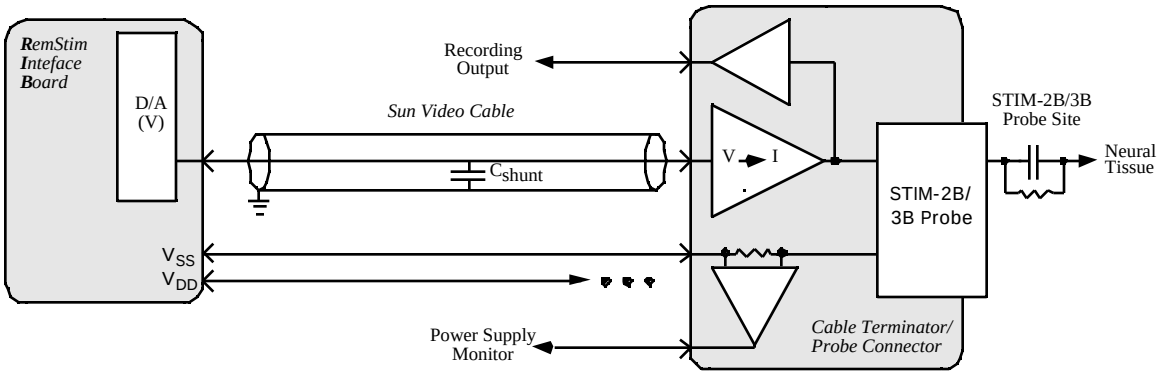


Fig. 12: This block diagram shows all of the various circuits that will be included on the cable terminator/probe connector and how they relate to the overall system.

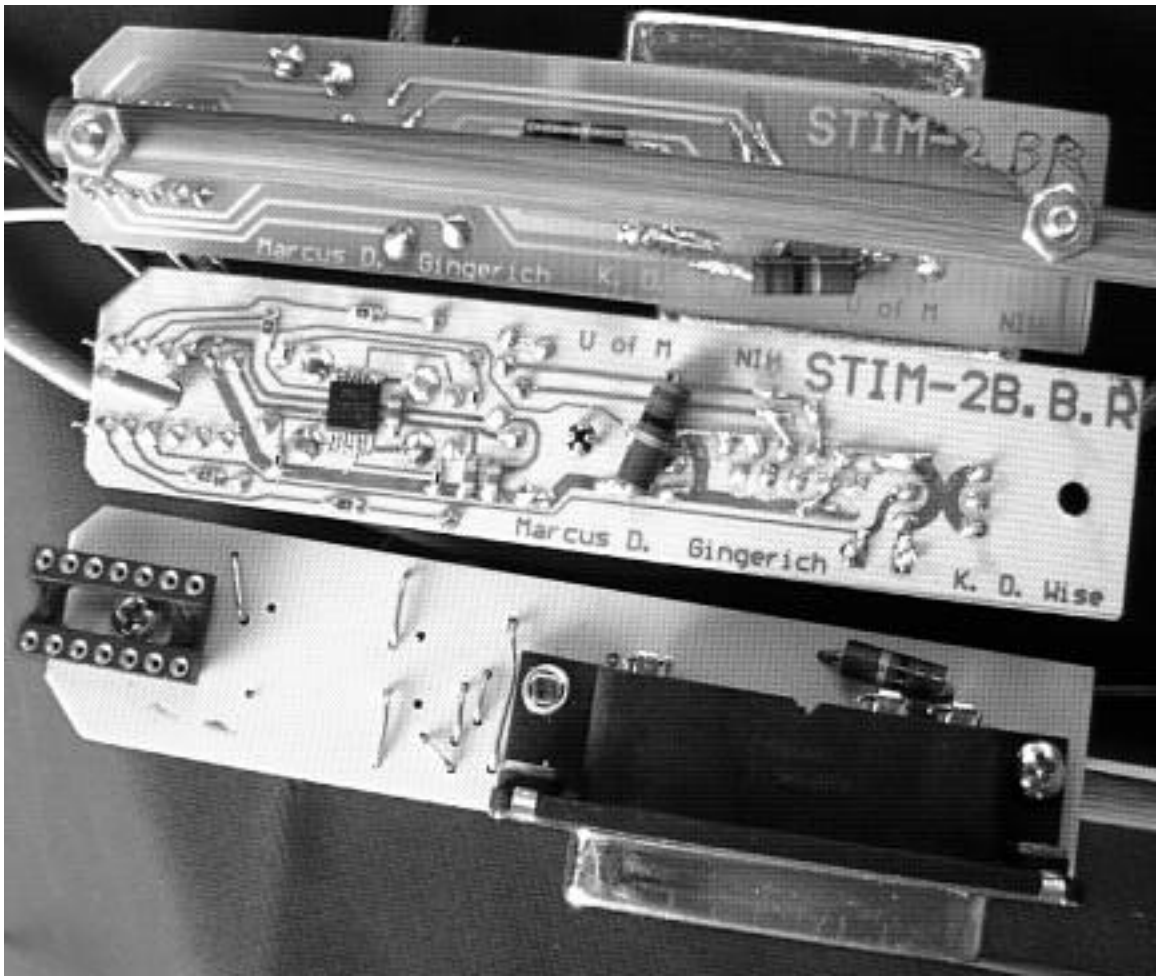


Fig. 13: A photograph of several versions of the cable terminator/probe connector PCBs. The upper connector has the micromanipulator mounting rod in place. The middle connector is has a unity gain buffer for recording through the stimulus channels of STIM-2B. The large resistors are the 75Ω cable termination resistors. The bottom PCB shows the other side with the cable connector and the dip socket which the probe stalks plug into.

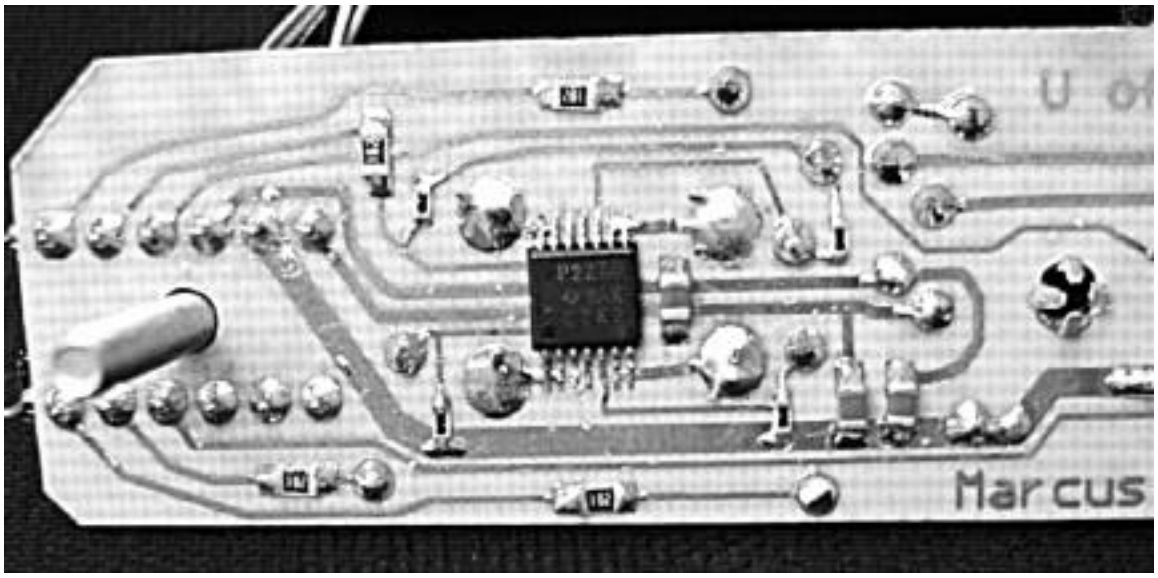


Fig. 14: This photograph shows a close-up of the four channel unity-gain buffer which is made up completely of surface mount devices to try to keep the size of the board to a minimum.

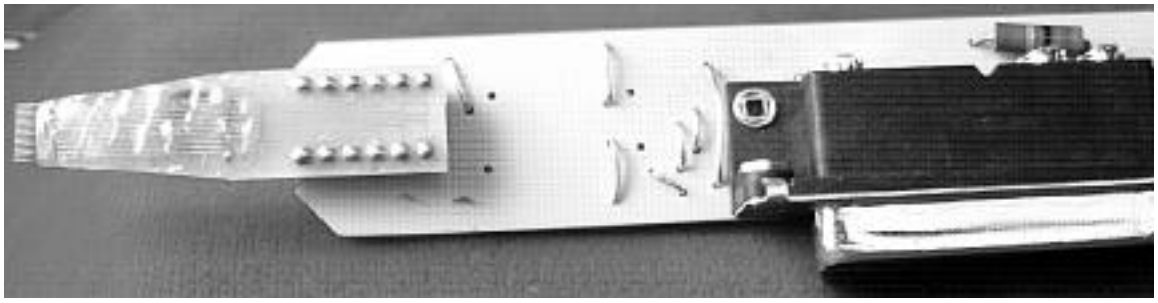


Fig. 15: A photograph of a PCB cable terminator/probe connector with the probe stalk mounted in the dip socket.

We are currently working on finishing a connector with all of the circuitry on a single PCB. We are also working on ways to improve the mechanical stability of the connector because our experience has shown that connector is somewhat flexible. Additionally, we are working on a reliable method of synchronizing the stimulation system with any recording system that is used.

In the coming quarter, we anticipate completing the final connector PCB and utilizing it in some experiments. We will continue to make improvements on the software as we identify problems or features which would make a probe users job easier. We also expect to continue the development of the software in the area of support for the remaining active probes, STIM-2/3. We also plan to complete the CV tests over a number of probes. The pulse testing will continue during the coming quarter. Finally, we plan continue using the STIM-2B probes in experiments which can use its capabilities and we plan to begin to use the STIM-3B probe arrays for some simple *in-vivo* experiments. Final design iteration of the circuitry for STIM-2 and STIM-3 is also beginning.

6. Conclusions

During the past term, we have continued a look at TiN as a possible stimulating electrode site. While for short pulses, it has an impedance comparable, and even slightly lower, than IrO sites of comparable size, it does not appear to provide superior performance overall. Some studies of both TiN and polypyrrole will continue in the recording area, however. Histology has been performed on probes implanted in guinea pig for three weeks. Immunocytochemistry with antibodies to cell specific proteins were utilized to improve our characterization. Glial Fibrillary Acidic Protein, a marker of glial cells, showed immunostaining around the probe tracts, confirming earlier observations that glial cells comprise an element of the tissue envelope. Vimentin immunoreactive labeling around probe tracks was also observed in many animals, indicating that epithelial cells can also be a component of the tissue envelope, likely due to cells migrating down the probe track from pia and/or pial cells pushed down during the probe insertion. Fibronectin (an epithelial matrix protein) immunolabeling was asymmetric on only one side of the probe and is being explored further.

With working versions of STIM-2B/3B now in hand, work is concentrating on the external interface required to operate them in detailed and automated mapping studies between the dorsal cochlear nucleus and the inferior colliculus in guinea pig. The goal is to be able to acoustically stimulate cells in DCN and record from the cells they drive in IC, then repeating this using electrical stimulation of the same cells in DCN. We have improved the graphical user interface (GUI) for the probes so that we can produce the multi-channel signals necessary to these mapping studies along with the required software. We have also developed the cabling and external drive circuitry to enable the experiments to proceed. We hope to carry out the first such experiments during the coming term. Circuit iteration on STIM-2 and STIM-3 is also now underway.